NITROGEN CONTENT AND DISTRIBUTION IN EGGS OF MELANOPLUS DIFFERENTIALIS DURING EMBRYONIC DEVELOPMENT [1]

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Introduction

During the past few years, attention has been given to studies on the physiology of the egg and embryo of the grasshopper, *Melanoplus differentialis*. Since most of this work has been based on the intact egg as a unit, a growth curve based on the total nitrogen as an index of the protein content seems desirable as a further basis of reference. A study has therefore been made to determine the nitrogen content of eggs of various ages and also the distribution of this nitrogen among shell, yolk and embryo.

Materials and Methods

The preparation of the eggs consisted in removing them from the pods in which they were laid and washing in distilled water to remove any adhering substances. They were kept at 25° C. until they had reached the desired stage or until they had entered the diapause, or blocked state (Slifer, 1932). In order to obtain postdiapause eggs of a known developmental stage, the eggs were subjected to cold (5° C.) soon after diapause began and kept at that temperature for several months. When the temperature is again raised to 25° C., they resume development and hatch in 18 or 19 days. Before analysis, samples of the eggs were examined after dissection to make sure that all were in the desired developmental stage.

For the first part of the study, the original Kjeldahl method for total nitrogen determination as outlined in Hawk and Bergeim's "Practical Physiological Chemistry" (but using a smaller apparatus) was used on lots of about 50 diapause eggs. Some of the lots contained eggs from one pod only, while others were obtained by mixing eggs from 3, 4, or 5 pods. These mixtures gave a value nearer the average, as the eggs of one pod tend to vary less widely than eggs from different pods.

The reagents used were a digestion mixture of 3 grams Cu_2SO_4 and

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1 gram K_2SO_4 in 300 cc. concentrated H_2SO_4; concentrated NaOH solution, for neutralization; 0.0706 N HCl (1 cc. $\equiv$ .9886 mg. N), for receiving the NH_3; and 0.1074 N NaOH, for back titration. The amounts used were about 15 cc. of the digestion fluid, sufficient NaOH to provide an excess of alkali after neutralization of the acid, and approximately 10 cc. of HCl (added from a stopcock burette). Back titration was done with a 50 cc. Mohr burette and with phenolphthalein as an indicator.

The larger part of the work was done by means of Keys' modification of the Kjeldahl technique for rapid microanalysis (Keys, 1940). With this modification, quantities of 0.01–20.0 mg. of total nitrogen per sample may be analyzed. The digestion was carried out by a mixture of Cu_2SO_4, K_2SO_4, and H_2SO_4 as in the previous Kjeldahl method and phenolphthalein was used as an indicator. These are not the reagents used by Keys, but the change does not appreciably affect the accuracy of the method. The HCl was measured into the receiving tube from a .2 cc. pipette graduated in hundredths and .2, .3, or .4 cc. of the acid were used depending upon the sample to be analyzed. The acid was the same as in the previous method (1 cc. $\equiv$ .9886 mg. N) but the NaOH was 0.10025 N. One to 5 cc. of the digestion mixture were required. Titration was done by means of a burette made from a similar .2 cc. pipette fixed with a 1 cc. syringe according to the principle of the Linderstrøm-Lang pipette (Glick, 1935). It can be easily controlled and read to one thousandths of a cc.

Single eggs were analyzed and in addition determinations were run on embryos, shell and yolk separately. The embryos were dissected out and washed, the shells washed, and the washings and yolk combined. Each part was then analyzed separately. For the earlier stages 2 embryos were analyzed since one contains too little nitrogen for accurate determination. A complete series of stages both before and after diapause were analyzed by this procedure. Until the seventh day after laying, the embryos are too small to handle, so no determinations were possible on these younger stages. In late postdiapause eggs, after the yolk mass was completely engulfed by the embryo (about 7 days postdiapause), the unabsorbed yolk was pressed out of the embryo and analyzed separately although some workers include it with the embryo. In all cases, 1-cc. samples of a known solution (1 mg. NH_4Cl in 10 cc.) were analyzed after every 3 to 5 determinations as a check on the reagents and apparatus. If the error exceeded 2 per cent, the determinations done just previously were discarded.

Accuracy

Macrodeterminations were used only when the results on known samples were within 5 per cent of the known value. This error is rather large, but even using 50 eggs the quantity of nitrogen is close to the lower

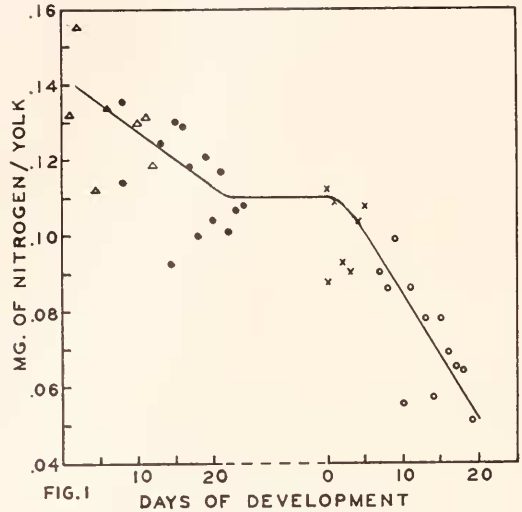

FIG. 1

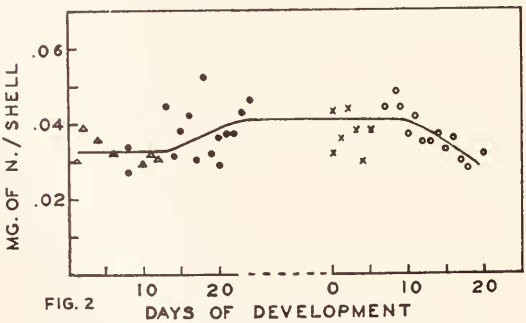

FIG. 2

Fig. 1. Yolk nitrogen. Each point indicates one determination and each pod is represented by a different symbol. The diapause period, which lasts for several months, is shown by the dotted portion of the abscissa. The level of the curve during this period was determined from an average of several analyses.

Fig. 2. Shell nitrogen. The symbols are the same as in Fig. 1.

limit for accurate determination by this method, and too many eggs would have been required to obtain greater accuracy. However, variations between lots from different pods were as much as 20 per cent so that the error in determination is not too great for statistical study.

All determinations of known solutions done by microanalysis were

within 2 per cent of the theoretical value or the experimental analyses just preceding were discarded. The control values ranged between 101.8 per cent and 98.1 per cent but 99.8 per cent and 100.8 per cent were the values more often found. These values correspond to burette readings of 69 and 70 mm.^{3} while the extremes used were 68 and 71. The 2 per cent accuracy is all that is necessary since individual variation may be as much as 20 per cent between diapause eggs from different pods.

Results

Macrodeterminations were used to establish the value for the total nitrogen of the intact diapause egg. The eggs are quite variable and therefore a wide range of nitrogen values even for eggs from the same pod exists. The variation is even greater when different pods are used. For this reason, it was thought advisable to use the results of the macro-

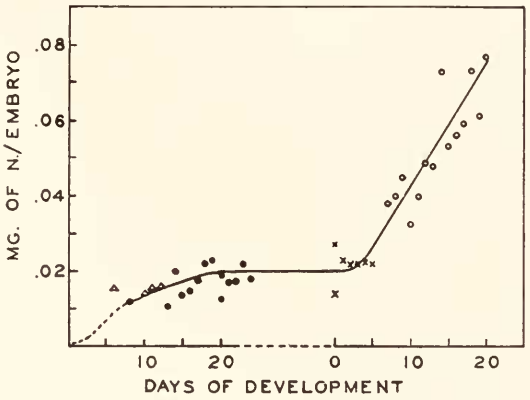

Fig. 3. Embryo nitrogen. Symbols as in the preceding graphs.

analyses to determine the statistical average. The value assigned (174 γ) for the diapause is the average of 18 determinations of 50 eggs each. This serves as a check on the other curves made from micro-determinations on fewer pods.

It will be seen from the yolk curve (Fig. 1) that a wide variation in the yolk nitrogen accounts for most of the variation of the egg as a whole. This can be seen by comparing the individual points on the graph of the yolk (Fig. 1) with those on the curve plotted by adding together the 3 parts of the egg (Fig. 4). In spite of the rather wide variation, a definite trend downward may be seen. The yolk nitrogen drops from about 80 per cent of the total nitrogen at laying to about 60 per cent at diapause. During diapause it remains constant and when development is resumed it decreases rapidly from 110 γ at diapause to 50 γ at hatching.

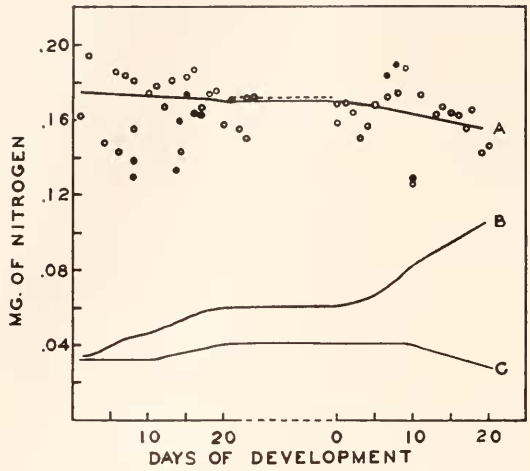

FIG. 4. A composite of the three preceding graphs No individual determinations were used but the three smoothed curves were added. *C*, shell *N*; *B*, shell and embryo *N*; *A*, shell plus embryo plus yolk *N*. The ordinate length beneath curve *C* is a representation of the shell nitrogen; that between *B* and *C*, the embryo nitrogen; and between *A* and *B*, the yolk nitrogen. The level of the broken line is the level for diapause determined by the macromethod.

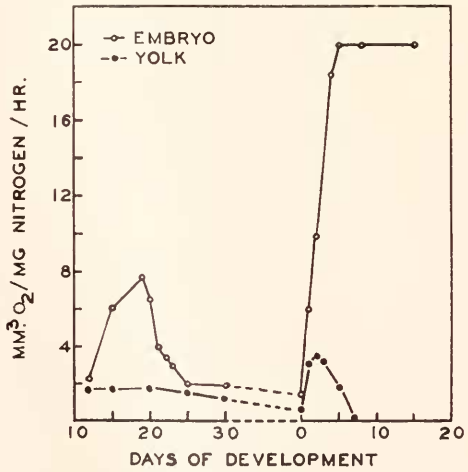

FIG. 5. Curves obtained by dividing the O_2 consumption curves of Boell and Bodine (1936) by the embryo and yolk *N* curves of this paper, showing the changes in basal respiration, i.e., mm.^{3} O_2/mg. nitrogen/hr., during embryonic development.

The shell nitrogen (Fig. 2) was found to be about 30 γ per egg until about the tenth day. Between the tenth and twentieth days it rose to more than 40 γ per egg, which was maintained during the diapause period. Between 5 days postdiapause and hatching, the shell nitrogen decreased to about 35 γ.

The curve for embryo nitrogen (Fig. 3) rises steadily during prediapause development, is level at diapause, and again rises rapidly in the postdiapause period.

Figure 4 is a composite of the three preceding graphs. The points plotted for the nitrogen of the whole egg were found by analyzing whole eggs (solid symbols) and by adding the values found for embryo, shell, and yolk of the same eggs (open symbols). These points are extremely scattered and no curve can be drawn accurately based on these points. The curve drawn was made by adding the smoothed curves of the separate parts. The diapause level thus determined is not significantly different from the level taken from the average of the macrodeterminations on diapause eggs.

Discussion

The curve (Fig. 4) for the nitrogen of the whole egg is too indeterminate for any accurate quantitative analyses of the nitrogen behavior but it can be seen by the addition curve that no significant change, either increase or decrease, in nitrogen during the embryonic period occurs. The organism seems incapable of utilizing atmospheric nitrogen.

The early rise in shell nitrogen, as well as the drop in yolk nitrogen, corresponds to the formation of the white cuticle which begins at about the tenth day (Slifer, 1937). The loss of nitrogen from the shell in the postdiapause period is doubtless due to the digestion of this layer preparatory to hatching (Slifer, 1937).

The embryo nitrogen curve is comparable in shape to curves for various physiological activities of the embryo. A mm.^{3} O_2/mg. embryonic N/hr. curve has been made (Fig. 5) by dividing the mm.^{3}/embryo/hr. curve of Bodine and Boell (1936) by the mg. N/embryo curve of this paper, which may be used as a basal curve for future work. The rate of O_2 consumption per mg. of embryo nitrogen rises steadily in prediapause, but drops rapidly at the onset of diapause, the diapause level being 1.7 mm.^{3} O_2/mg. N. As soon as development resumes the rate rises again, until, on the fifth day, it reaches 20 mm.^{3} O_2/mg. N where it remains practically constant throughout the remainder of the embryonic period.

During prediapause the O_2 consumption of the yolk (Fig. 5) is a constant value, 1.8 mm.^{3} O_2/mg. N. It drops during diapause to .5

mm.^{3} In the postdiapause period, it rises to a peak of 3.7 mm.^{3} in 2 days and then drops rapidly to zero when the yolk has been engulfed by the embryo.

Summary

The total nitrogen content of the egg of the grasshopper, *Melanoplus differentialis,* has been determined and also the changes in its distribution among shell, embryo, and yolk. The nitrogen content of the whole egg is constant while the embryo nitrogen increases at the expense of the yolk nitrogen. The changes in shell nitrogen correspond to the formation and digestion of the cuticle. No change occurs during diapause.

A basal metabolism curve is computed from the embryo nitrogen curve of this paper and the oxygen consumption curve of Boell and Bodine (1936) which shows that the oxygen consumption rate rises to 20 mm.^{3} O_2/mg. N at 5 days postdiapause and remains steady at that value. A similar curve is computed for the yolk. The metabolic rate is constant in the prediapause at a value of 1.8 mm.^{3} O_2/mg. N and in the postdiapause drops from a peak at 2 days to 0 at 10 days. Both curves show very low rates during diapause.

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